Brain Research, 413 (1987) 374–378
Elsevier

BRE 22313

Localization of a developmentally regulated neuron-specific protein S54 in dendrites as revealed by immunoelectron microscopy

Tomoaki Shirao[1], Hiroshi K. Inoue[2], Yumiko Kano[3] and Kunihiko Obata[1]

Departments of [1]Pharmacology, [2]Neurosurgery and [3]Anatomy, Gunma University School of Medicine, Maebashi (Japan)

(Accepted 10 March 1987)

Key words: Nervous system-specific protein; S54 protein; Dendrite; Synapse; Immunoelectron microscopy; Monoclonal antibody

We sought to determine the ultrastructural localization of the developmentally regulated neuron-specific protein S54 in the chicken cerebellar cortex and optic tectum. The brains were fixed by perfusion with paraformaldehyde and glutaraldehyde. Frozen sections were immunocytochemically labeled with a monoclonal antibody to S54 protein. The immunoreactivity for S54 protein was localized in dendrites. No immunoreactivity for S54 protein was detected in axons and their presynaptic terminals.

It has been suggested that certain proteins are distributed unevenly within neurons and have an important role in each compartment[3]. Recent studies have shown that some proteins in brain are restricted not only to certain cell types but also to particular compartments of the neuron[3,6,7,9,10,12]. A high-molecular-weight microtubule associated protein (MAP2)[1,21] type-II cyclic-AMP-dependent protein kinase[13] and calmodulin[22] are highly concentrated in dendrites. On the other hand, certain isotypes of α-tubulin[5,20] and another high-molecular-weight microtubule associated protein (MAP3)[8] are highly concentrated in axons.

It has recently been reported that proteins S5, S6 and S54 (mol. wts. 95 kDa, 100 kDa and 110 kDa, respectively) have a high degree of structural homology and are expressed characteristically at certain developmental stages in the chick brain[18,19]. Proteins S5 and S6 appear temporarily at the developmental stages corresponding to the migration of neurons and the growth of neuronal processes, respectively. They are present in both fiber tracts and neuropil in all brain regions at certain embryonic stages, but they are not detected in the adult brain. On the other hand, S54 protein appears in parallel with further maturation of the nervous system, and is present in the adult brain. S54 protein is a neuron-specific protein and is enriched in neuropil but not in fiber tracts[19]. It has been suggested that S54 protein is localized at synaptic junctions[19]; however, it has not yet been determined whether S54 protein is present in axonal terminals or postsynaptic elements. In this study, we performed immunoelectron microscopy of S54 protein on cerebellar cortex and optic tectum, which have been analyzed by light microscopy in our previous paper[19], in adult hens, and disclosed the localization of S54 protein in dendrites.

Anesthetized hens (5 months old) were perfused transcardially with 4% paraformaldehyde and 2% glutaraldehyde in phosphate-buffered saline. The cerebella and optic tecta were excised and fixation continued in the same fixative at 4 °C for 12 h. For light microscopy, tissues were embedded in paraffin and stained with monoclonal antibody (MAb) M2F6 as described previously[19]. For electron microscopy, tissues were incubated for more than 12 h in 30% sucrose in phosphate-buffered saline. Frozen sections 12–14 μm in thickness were then cut on a cryostat microtome. Some tissues were cut into sections of 30 μm thickness on a Vibratome. The sections were then

Correspondence: T. Shirao. Present address: Division of Neurobiology, Cornell University Medical College, 411 East 69th Street, New York, NY 10021, U.S.A.

incubated with MAb M2F6 and a 1:250 dilution of horseradish peroxidase-conjugated goat anti-mouse IgG (Cappel) for 3 h each. After reaction with diaminobenzidine tetrahydrochloride, the sections were postfixed with 1% osmium tetroxide, dehydrated through ethanol series, and embedded in Epon. Ultrathin sections were cut with an ultratome, counterstained with uranyl acetate and lead citrate, and examined with an electron microscope[14].

The manufacture and characterization of MAb M2F6 used in this study have been previously described[19]. This MAb was obtained as culture supernatant of the hybridoma cells. It reacts specifically with all of proteins S5, S6 and S54. Since S5 and S6 proteins are not detected in the adult brain either with two-dimensional gel electrophoresis[18] or immunoblot analysis[19], the protein which reacted with MAb M2F6 in the present immunocytochemical study is S54 protein. Furthermore, specificity of the present immunocytochemical method was established by the substitution of antibody-free culture supernatant of X63-Ag8-653 myeloma cells or of MAb 171 B5 (ref. 14) that does not react with S54 protein for MAb M2F6.

By light microscopy, as previously reported[19], stainings of brain sections with MAb M2F6 showed a distinctive pattern in which a certain cellular element was reproducibly labeled. It appeared as a myriad of small punctate profiles, each $<1.5\,\mu$m across (Fig. 1). In the cerebellar cortex, it was present in some but not all of the cerebellar glomeruli (Fig. 1A), which are complex synaptic arrangements consisting mainly of mossy fiber terminals and granule cell dendrites[15]. Immunostaining was hardly observed in the molecular layer. Purkinje cells and white matter were completely unstained (data not shown). In the optic tectum (Fig. 1B) the immunoreactivity for S54 protein was widely distributed except for the ependyma and the layer of the retinal fibers (so in Fig. 1B). The small stained profiles were most abundant and in-

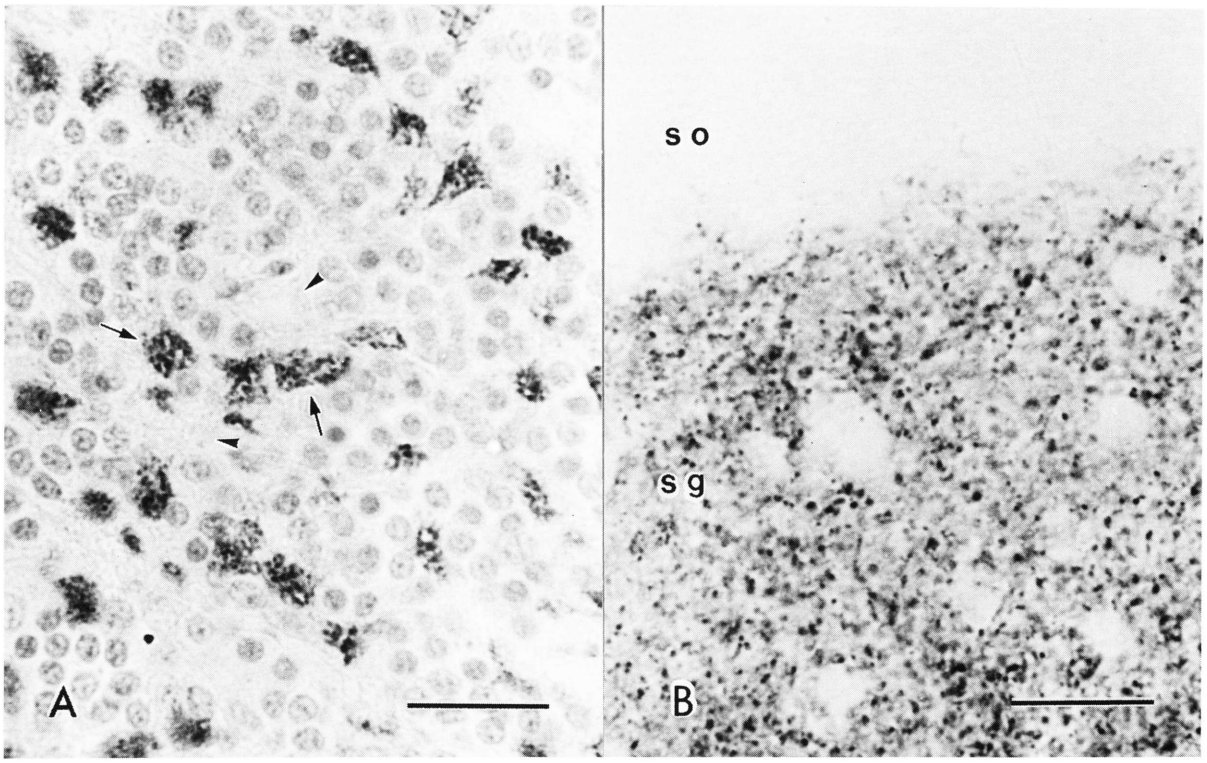

Fig. 1. Light micrographs of the granular layer in the cerebellum (A) and of superficial layers in the optic tectum (B) stained with MAb M2F6. A: intensely stained punctate profiles are present in the cerebellar glomeruli (indicated by arrows). Hardly stained cerebellar glomeruli (indicated by arrowheads) are also observed. Bar = $20\,\mu$m. Hematoxylin counterstain. B: intensely stained punctate profiles are visible in the stratum griseum et fibrosum superficiale (sg). There is no immunostaining in the stratum opticum (so), which is a retinal fiber tract. Bar = $20\,\mu$m. No counterstain.

tense in the superficial laminae of the stratum griseum et fibrosum superficiale (sg in Fig. 1B). The identification of these stained profiles was pursued further in electron microscopy.

The immunostaining pattern of the granular layer in the cerebellum, by electron microscopy, confirmed the light microscopic observation of reaction product for S54 protein in the cerebellar glomeruli. Furthermore, the reaction product was situated in the postsynaptic cytoplasm of dendrites facing the large mossy fiber terminals but not in the mossy terminals themselves (Fig. 2). These labeled dendrites are thought to be granule cell dendrites. Since light microscopy showed that there was no visible immunostaining in the axons in the molecular layer (data not shown) and in the cell bodies in the granular layer, immunoreactivity for S54 protein is thought to be restricted in dendrites within a granule cell.

In the neuropil of the optic tectum, reaction product for S54 protein was found in cell processes. These labeled processes were not myelinated axons. Many of these labeled processes were postsynaptic to unlabeled presynaptic terminals and thus appeared to be dendrites (Fig. 3). However, we could not rule out the possibility that some of the smaller labeled processes were unmyelinated axons. The density of the immunoreactivity appeared to be greater in the postsynaptic region than in other regions of dendrites (Fig. 3A). The reaction product in the cytoplasm of dendrites was amorphous or flocculent both in the cerebellar cortex and the optic tectum, but due to limitation of the present technique the association with any particular organelle could not be determined. There was no detectable reaction in the glial cell elements nor axonal presynaptic terminals.

The results of this study indicate that in the neuropil S54 protein is localized in dendrites and that the highest concentration of S54 protein occurs in the postsynaptic region of dendrites under the present set of conditions. It suggests that S54 protein is trans-

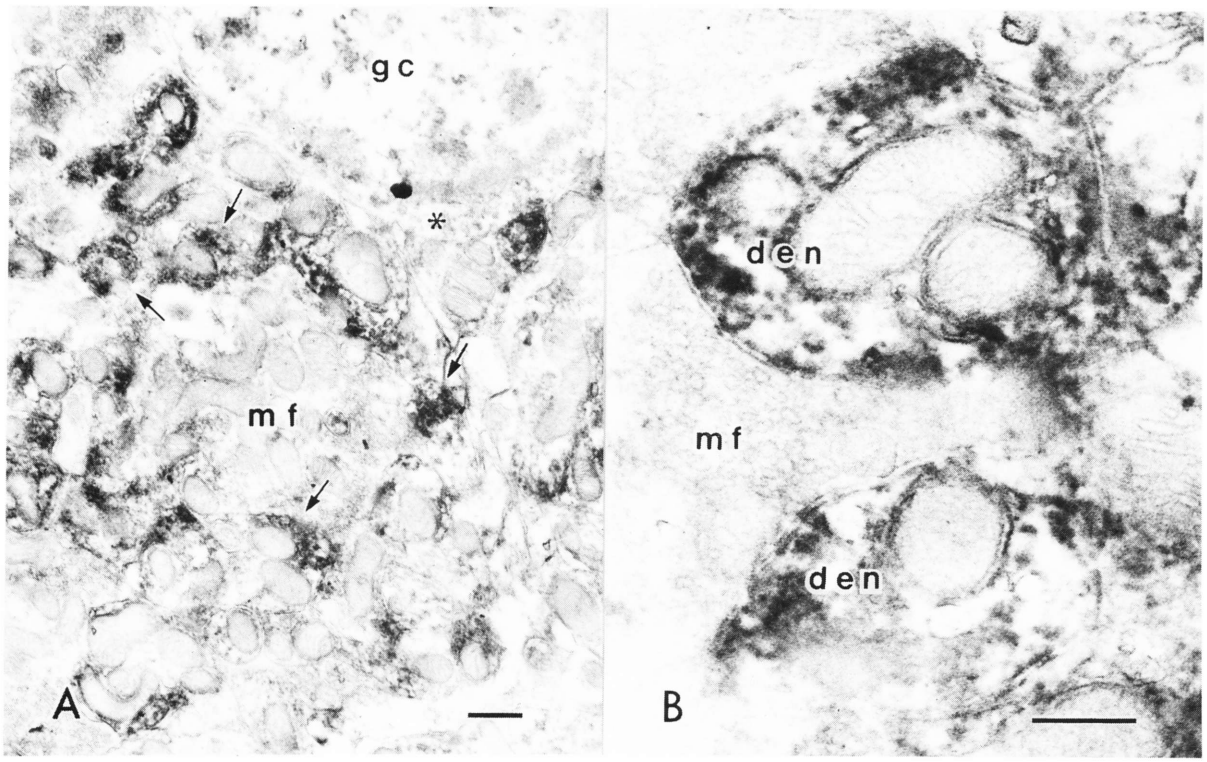

Fig. 2. Low (A) and high (B) magnification of electron micrographs derived from a section cut on a Vibratome showing the localization of the immunoreactivity for S54 protein in dendrites of the cerebellar glomeruli. A: reaction product is observed in the dendrites facing the mossy fiber terminal (mf). Note the absence of reaction product in the granule cell somata (asterisk) and presynaptic terminals of mossy fibers (mf). gc: granule cell nucleus. Bar = 0.5 μm. B: amorphous reaction product for S54 protein is present in the postsynaptic cytoplasm of dendrites (den), but absent from the presynaptic terminal (mf), which contains synaptic vesicles. Bar = 0.25 μm.

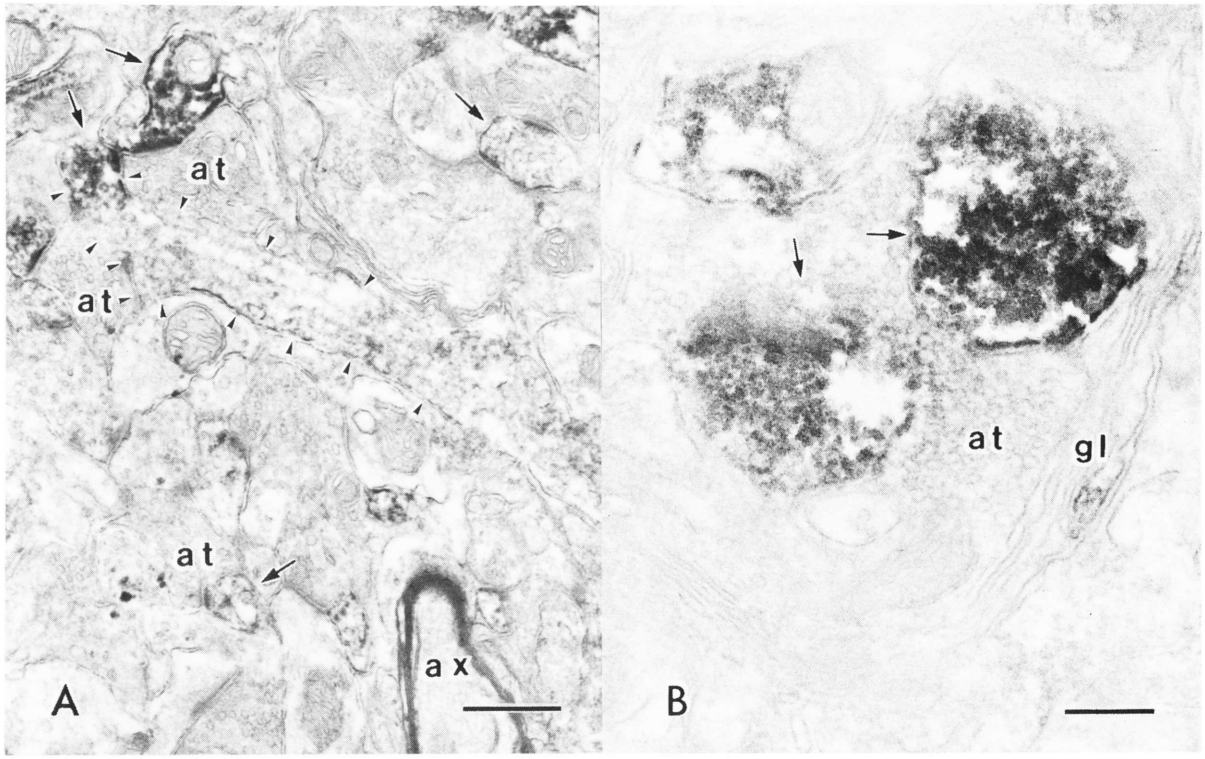

Fig. 3. Low (A) and high (B) magnification of electron micrographs derived from a frozen section showing the localization of the immunoreactivity for S54 protein in dendrites of the stratum griseum et fibrosum superficiale of the optic tectum. Reaction product is observed in the postsynaptic cytoplasm in dendrites (indicated by arrows) facing the unlabeled presynaptic terminal (at) and in the dendritic shaft (outlined by arrowheads). No peroxidase reaction occurs in the myelinated axon (ax), the presynaptic axon terminals (at) and the glial elements (gl). A: bar = 0.5 μm. B: bar = 0.25 μm.

ported from the soma of neurons preferentially to the dendrite but not to the axon, and finally concentrated in the postsynaptic regions of the dendrite. Whether or not S54 protein is associated with any intracellular organelle cannot be determined until a more discrete label than the present technique, such as immunogold labeling, is used. It is thought that S54 protein in the soma and the axon is too low in concentration to be detected in our present condition. However, we cannot exclude the possibility that some special circumstances interfere with immunostaining of axonal S54 protein.

Proteins S5, S6 and S54 are purportedly concerned with cell migration, neurite growth and synaptogenesis during development[18,19]. In the adult nervous system S54 protein may be involved in modification of the structure of dendrites. Dendrites are enriched with cytoskeletal proteins[1,11,21], type-II cyclic-AMP-dependent protein kinase[13] and calmodulin[22]. Cyclic AMP and calmodulin are the major intracellular regulatory signals[2]. Therefore the structure and function of the dendrite may be far more dynamically controlled than those of the axon. Furthermore, it has recently been reported that the dendrites of sympathetic ganglion cell appreciably change their arborization in young adult mammals[16,17]. The light microscopic observations have shown that a portion of neurons, such as Purkinje cells, lack the immunoreactivity for S54 protein in their dendrites. It will be of interest in future investigations to elucidate the types of dendrites in which S54 protein is concentrated and to determine whether S54 protein-rich regions are subject to growth and other plastic changes.

We wish to thank Professor H. Ishikawa for critical and stimulating discussion during the course of this research. This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.